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ISIS 3521/CGP 64128A- Antisense to Protein Kinase C-Alpha.

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The overexpression of protein kinase C-alpha (PKCα), a cytoplasmic serine-threonine kinase involved in signal transduction, has been demonstrated to play a role in the development and maintenance of several tumor types. ISIS 3521 (CGP 64128A) is a phosphorothioate antisense oligodeoxynucleotide, 20 bases in length, which is complementary to a sequence in the 3'-untranslated region of human PKC-α mRNA. It inhibits PKC- mRNA expression in a dose and sequence dependent manner. The extent of PKC- mRNA inhibition correlates with antitumor activity in human tumor xenograft models. Two Phase I studies are in progress. In CS-1, ISIS 3521 is administered as a 2-hour infusion, three times per week for 3 weeks followed by one week of rest. Thirty patients with a variety of refractory malignancies have been treated with doses ranging from 0.15 mg/kg/day of dosing to 5.0 mg/kg/day of dosing. There have been no dose-limiting toxicities and only transient prolongation of aPTT has been observed. One patient with refractory low-grade lymphoma which had been documented to progress pre-study has had a minor response and remains on study after 6 cycles (6 months) of treatment. In CS-2, ISIS 3521 is administered as a 21-day continuous infusion, repeated every 4 weeks. Fifteen patients with various tumor types have been treated with doses ranging from 0.5 mg/kg to 3.0 mg/kg. Transient grade 2 thrombocytopenia has been observed in 2 patients and grade 1 leukopenia has been observed in 1 patient. There has been no other drug related toxicity. One patient with refractory ovarian cancer has developed central necrosis of her abdominal mass and a 40% decrease in bidimensionally measurable disease. Accrual and dose escalation continue in both trials. Samples for pharmacokinetic analyses have been obtained from each patient on each trial. At 4 mg/kg on CS-1, the C_{MAX} is 14.5 μg/ml (range 13.4 – 15.5). In contrast to monkey PK, there has been a less than linear dose-response relationship with C_{MAX}. The $t_{BD}a$ at 4.0 mg/kg is 105 minutes compared to 67 minutes at 3.0 mg/kg and 35 minutes at 0.30 mg/kg.

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INTRODUCTION OF NOVEL T-CELL RECEPTORS INTO T CELLS FOR ADOPTIVE IMMUNOTHERAPY.

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We have developed a system for rapid generation of retroviral supernatants capable of high efficiency transduction of $CD8^+$ T lymphocytes and $CD34^+$ hematopoietic progenitors. This approach enables rapid generation of retroviral supernatants from many constructs simultaneously, each capable of transduction of primary human cells without the time consuming need to generate stable producer clones for each retroviral construct. *Kat* retroviral vectors and packaging plasmids were cotransfected into tsA54 cells, followed by retroviral harvest 48 hrs post transfection. Viral titers of 10^6 -2 x 10^7 have been obtained when assayed on 3T3 cells. High titer pseudotyped viral stocks have been prepared incorporating envelopes from Eco, Ampho, Xeno and 10A1 using this approach. We consistently achieve transduction frequencies of 20 - 70 % for primary human $CD8^+$ T lymphocytes and 20-70% for mouse, primate or human $CD34^+$ hematopoietic progenitors. This system eliminates the need for co-cultivation of producer and target cells followed by purification of target cells.

In addition we have developed an amphotropic retroviral packaging clone (Puzi*kat*) in 293 cells that, in contrast to previously described systems, stably expresses gag, pol and amphotropic envelope for 6 weeks without selection. Transiently, Puzi*kat* gives 5 to10-fold higher viral titers compared to the 3T3-based amphotropic packaging lines. Retroviral producer clones developed from Puzi*kat* have yielded supernatant titers of 1-3 x 10^7 /ml for frozen supernatants assayed on NIH 3T3 cells, and are also stable for 6 weeks in culture without selection. Transduction of $CD8^+$ primary human lymphocytes with supernatants derived from these 293 producers has resulted in transduction levels from 30-95%, 5 to 10-fold greater than reported in the literature for supernatant infection and equivalent to or greater than those previously reported for co-cultivation of T cells with producer clones.

Viral supernatants produced by Puzi*kat* and its producer clones are free of detectable replication-competent retrovirus by extended cultivation with M. dunni cells, followed by a PG4 assay.

The highly efficient RCR-free system described here should have significant advantages for clinical gene therapy applications in adoptive immunotherapy with genetically modified T lymphocytes.

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ANTISENSE AND DOMINANT NEGATIVE CONSTRUCTS AS SELECTIVE INHIBITORS OF PKC ISOZYMES

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There is general agreement that enzymes of the PKC family are major players in a broad variety of signalling pathways including regulation of cellular proliferation, differentiation, structure, apoptosis, hormone action etc. Although, in cell-free systems, PKC isoforms phosphorylate a variety of proteins, the precise function of the various isozymes in vivo has remained obscure. In order to elucidate the biological role of the different PKC-isotypes, antisense constructs and dominant negative negative mutants have been employed. Since some PKC isozymes are essential for cellular proliferation or induction of programmed cell death, these agents may also lead to new antitumor agents. With regard to antisense, strategies based on hydrolysis-resistant ODNs and vectors encoding antisense RNA were follwed. Exposure to PKC alpha directed antisense phosphothioate ODNs were found to reproduce PKC alpha levels to 20% of controls within 48 h at 1 μM. 50% of the treated cells were apoptotic. The same concentration of the sense construct exhibited only a marginal effect on cell viability. Transient transfections with constructs encoding antisense PKC epsilon and antisense zeta RNA - not however antisense alpha- abrogated the Ras-induced transcriptional activation of c-fos. The implication of PKC epsilon and zeta in Ras-mediated Fos-induction could be confirmed by employing dominant negative mutants of the corresponding isotypes. Dominant negative mutants were generated by introducing point mutations in the ATP-binding-site of the catalytic domain of the enzymes. Compared to antisense constructs dominant negative mutants offer some advantages mainly because they counteract the existing enzyme level, whereas the antisense effects require a depletion of the endogenous enzyme which-depending on the half life of the corresponding protein- may take considerable time during which the antisense construct has to remain present at sufficiently high concentrations. Conclusion based on the effects of dominant negative mutants were confirmed by constitutively active forms produced by introducing point mutions in the pseudosubstrate domains of the enzymes. Some recent results obtained with dominant negative and constitutively active mutants of PKC isotypes alpha, epsilon, zeta, lambda and zeta will be discussed.

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MOLECULAR STRATEGIES TO GENERATE TUMOR VACCINES FOR NON-HODGKIN'S LYMPHOMA.

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Despite the use of intensified chemotherapy and radiotherapy, the majority of patients with low grade non-Hodgkin's lymphoma remains incurable. The relatively low effectiveness of these modalities and their potential toxicity has inspired the search for alternative therapeutic strategies. One such approach attempts to utilize the immune system to target and eliminate lymphoma cells specifically. Lymphoma are unique in that they express tumor-specific antigens, the idiotypic determinants of the immunoglobulin variable regions. These offer the opportunity to create lymphoma-specific vaccines. However, the use of idiotypes (Id) for tumor vaccination has been hampered by the relatively low immunogenicity the Id sequences. Therefore, several strategies might enhance the efficacy of anti-Id vaccines: 1. Use of professional antigen-presenting cells (APCs) such as dendritic cells (DCs) loaded with Id peptide sequences; 2. gene transfer of cytokines or costimulatory molecules into lymphoma cells; 3. stimulation of CD40 present on most lymphoma cells by CD40 ligand (CD40L) which induces the up-regulation of costimulatory and MHC molecules on B cells. 4. Use of monoclonal or bispecific antibodies detecting lymphoma specific epitopes. It is open which of these strategies (or a combination thereof) will be optimal. Our group has developed simplified strategies for the isolation of lymphoma-specific idiotypes, and a vector system based on adeno-associated virus (AAV) allowing to express costimulatory B7 molecules on lymphoma cells. With this recombinant AAV vectors coding for human B7-1 and B7-2 genes, we transduced different lymphoma cell lines. B7-1 and B7-2 transduced tumor cell lines induced a strong anti-lymphoma T-cell response as shown by the enhanced proliferation, cytokine (IL-2) production and a cytotolytic T cell (CTL) response *in vitro*. Stimulation of freshly isolated lymphoma cells with CD40L also induced an up-regulation of many co-stimulatory molecules including B7-1 and B7-2, and stimulated a strong CTL response *in vitro*. These studies illustrate that lymphoma cells have apparent defects in costimulation which can be corrected to create a specific cytolytic T cell activity against the tumor.